Result: MiRNA-21-5p and miRNA-24-3p levels were high throughout hypoxia and reoxygenation. Single blockade with anti-miRNA-21-5p resulted in a significant increase in its downstream target SOD2 ($P<0.05$). Anti-miRNA-24-3p treatment resulted in no change in either of its downstream targets, HMOX1 or SOD2. This was reflected in the failure of dual blockade to produce a synergistic effect on the shared target, SOD2.
Conclusion: Anti-miRNA-21-5p results in a significant increase of SOD2, which is well characterised as protective during IRI. Anti-miRNA-24-3p appears to have no effect on PTECs, contrary to previous work in endothelial cells, perhaps suggesting a cell specific response of microRNAs. Normothermic machine perfusion could be used to deliver dual ASOs; allowing simultaneous targeting of different kidney cell types.
Take-home message: The delivery of anti-miRNA-21-5p therapy pre-transplant, using normothermic machine perfusion, has the potential to reduce ischaemia reperfusion injury and improve kidney transplant outcomes.

O25

TARGETING THE RENAL TUBULAR EPITHELIUM WITH ANTI-MIRNA THERAPY: A POTENTIAL MECHANISM FOR MINIMISING ISCHAEMIA REPERFUSION INJURY

E Irwin[1], E Thompson[1], S Tingle[1], P Ezuma[1], L Matthews[1], L Bates[1], V Shuttleworth[2], S Ali[2], N Sheerin[2], C Wilson[1]

[1]*NIHR Blood and Transplant Research Unit, Institute of Transplant, Freeman Hospital*
[2]*Institute of Cellular Medicine, Newcastle University*

Presenting Author Email: ellie@irwins.me.uk
Senior Author Email: Colin.Wilson@nuth.nhs.uk

Introduction: Ischaemia reperfusion injury (IRI) is an unavoidable, significant consequence of renal transplantation. MicroRNAs are small, non-coding RNA molecules that regulate multiple downstream mRNA targets. MiRNA-21-5p and miRNA-24-3p have been previously implicated in IRI. Antisense oligonucleotides (ASOs) block specific microRNAs, with previous work by our group demonstrating their delivery to kidneys using normothermic machine perfusion. Imaging these kidneys revealed ASO localisation around proximal tubule epithelial cells (PTECs). This project aimed to characterise ASO blockade against miRNA-21-5p and miRNA-24-3p in PTECs.
Method: HKC8 cells, a human PTEC cell line, were used throughout these experiments. Cells were placed in a hypoxic incubator for 24hrs, followed by 6hrs of reoxygenation to mimic IRI. HKC8s were transfected with ASOs using lipofectamine. RT-qPCR and Western Blots were used to evaluate expression of antioxidant targets, SOD2 and HMOX1.